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POLLEN PROVISION RECORDS FOR THREE SOLITARY BEE SPECIES OF *MEGACHILE* LATREILLE AND *HERIADES* SPINOLA (HYMENOPTERA: MEGACHILIDAE) IN SOUTHWESTERN MONTANA

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Abstract.—We identified the pollen included in nest provisions by three species of solitary bees at four sites in the vicinity of Bozeman, Montana. *Megachile relativa* Cresson and *Heriades carinata* Cresson were studied in trap nests in natural populations, whereas *Megachile rotundata* (F.) were from a managed, introduced population adjacent to an alfalfa field being used for seed production. Over 90% of 186 cells examined in the three species contained more than a single type of pollen (and up to seven different types). The most intensively studied species, *M. relativa*, provisioned with pollen from eight families of dicot plants (particularly Asteraceae and Fabaceae), as well as two unidentified monocots that were also common in provisions of *H. carinata* and *M. rotundata*. Results include new pollen records for all three species.

Key Words: *Megachile*, *Heriades*, trap nests, pollen provisions, alfalfa leafcutting bee

Pollen collected by adult female bees provides the major source of protein and other nutrients for their larvae, and the types of pollen collected can correlate with growth and survival during development (Guirguis and Brindley 1974, Schmidt et al. 1987, Horne 1995, Michener 2000). The types of pollen collected also affect the efficiency of bees as pollinators of cross-pollinated plants, a subject of particular interest to those managing bees for fruit or seed production (Free 1993). Knowledge of the types of pollen collected cannot always be determined from flower visitation records alone because bees may visit flowers solely to obtain nectar. For example, Hurd (1979) cited 16 families of plants whose flowers are visited by *Megachile relativa* Cresson and 8 families visited by *Megachile rotundata* (F.). However, after identifying pollen from nest cells, Strickler et al. (1996) identified two plant families used by *M. relativa*, and Stubbs et al. (1994) found four families used by *M. rotundata*. Although these discrepancies could be due to differences in pollen availability among sites, they may also reflect differences between nectar and pollen preferences. Thus, in order to determine the types of pollen collected by bees during actual pollen-collecting trips, it may be necessary to examine either the pollen present in nest provisions or that found on the scopae of foraging bees (or on bees in museum collections). Here, we report the pollen identified from nest cells of three megachilid bees, *M. relativa* and *M. rotundata*, and *Heriades carinata* Cresson, at four sites in the vicinity of Bozeman, Montana.

Materials and Methods

We identified pollen that we removed from the nest cells provisioned by bees during the summers of 1999 and 2000. *Megachile rotundata* nested within 0.5 × 9.5 cm deep tunnels in commercial polystyrene "bee boards" manufactured by Beaver Plastics (Edmonton, Alberta) for use in commercial seed alfalfa production (Richards 1984). The shelter containing the boards was located between two plots of alfalfa (*Medicago sativa* (L.)) being grown for seed on the Montana State University Post Farm, 3 km west of Bozeman, Gallatin County, Montana. Bees nesting in these boards were purchased as overwintering prepupae from Mennie Bee Farms Inc. (Parkside, Saskatchewan). All *M. rotundata* cells were provisioned by bees during July and August 2000 at a time when alfalfa was in bloom. The *M. relativa* and *H. carinata* were from native populations that nested in two types of trap nests. The first type consisted of pine boards into which we drilled 15 cm long holes and inserted paper straws with internal diameters of 3.2, 3.7, 4.6, 5.9, 7.5, 8.0, and 9.0 mm. *Megachile relativa* nested in 4.6–9.0 mm tubes, and *H. carinata* in 3.7 mm tubes. The second trap nest type, used only by *M. relativa*, consisted of pine boards with 15 cm long grooves (6.3 and 9.5 mm diameter) routed in the sides, which were then fitted with removable plexiglass sheets (3 mm thick) to provide a transparent surface for viewing nest contents and removing pollen. Nest boards of both types were mounted on fence posts (at heights of 1.5–2.0 m) adjacent to trees and with the nest holes facing southeast.

We placed the trap nests at three sites: 1) western Bozeman (WB), located on the western end of Bozeman on the Montana State University Horticultural Farm (nests placed within an abandoned ornamental tree farm surrounded by agricultural test plots and weedy fields); 2) southeastern Bozeman (SEB), located in a residential area 3.0 km from WB (nests placed within area that contained ornamental flowering plants and which was 150 m from a weedy industrial storage yard and 300 m from a wooded stream); and 3) Rocky Creek Farm (RCF), just east of Bozeman and 5.6 km east of WB (nests placed along the weedy border of a cultivated field and shaded by lilac, *Syringa vulgaris* (L.)) which was not in bloom while the bees nested.

We used two methods to obtain pollen samples from 186 nest cells, including 145 from 44 *M. relativa* nests (from WB and SEB), 26 from 19 *M. rotundata* nests (all from the Post Farm), and 15 from 11 *H. carinata* nests (5 from WB, 10 from RCF). The first method was to insert the wooden end of a cotton swab stick into the nest and twist it in the provision of the outermost cell while the female was away from the nest. The second method was to open nests in the lab, taking pollen either from uneaten provisions, or from frass left by the developing larvae (Strickler et al. 1996). From mid-May through August 1999, we also collected flowers within 200 m of the nests at approximately three-week intervals. We used this pollen to create a reference collection following the methods described by Moore et al. (1991) and Sawyer (1988) with slight adaptations described below.

We placed pollen extracted from each cell or plant into an Eppendorf tube with 2 ml of distilled water and one drop of safranin. After 24 h, we centrifuged the samples at 3,000 rpm for 5 min, poured the dye off, and resuspended the pellet in water for a second rinse. After a second centrifugation, we poured off the supernatant and resuspended the pellet in two drops of water. We then placed the sample on a slide where it was allowed to dry before mounting it in Euparal and sealing the slide with clear nail polish. To identify pollen, we first examined the entire slide under a Nikon phase contrast light microscope (40×). We then examined each type of pollen at high power (100×) for identification. By using pollen identification keys (Kapp 1969) and comparing pollen from nests with pollen in ref-

erence samples, we identified most dicot pollen grains to family and many to genus. We made no exact counts of each type of pollen in samples, but we did record general estimates of the proportions of different pollen types, which were sometimes unevenly distributed on slides due to clumping. However, we roughly estimated the relative frequencies of different pollen types on each slide, as 1%, 5%, or greater values to the nearest 10%. Rare pollen types represented by only several grains on a slide containing thousands of pollen grains were excluded from counts to reduce the possibility of recording pollen incidentally picked up by females on flowers or other sources.

We did not compare the frequency distributions of pollen types provisioned by different bee species because samples came from different sites. However, we did compare pollen types in different types of *M. relativa* samples: 1) WB vs. SEB samples and 2) samples from uneaten provisions vs. those from frass. We first determined if there was a significant (Pearson's) correlation between sample types in the number of cells containing each type of pollen (a significant correlation indicating similarity of the two samples). Where the correlation was not significant, we used 2×2 chi-square contingency table analyses (each with d.f. = 1) to test the null hypothesis that the proportions of cells with and without a particular type of pollen (e.g., thistle) were the same in the two sets of samples (a significant difference indicating that a particular type of pollen was over- or under-represented in a set of samples).

Results and Discussion

Overall, the three species provisioned with pollen from nine families of dicot plants and two types of monocots (Fig. 1). Using reference samples, we distinguished at least three types of Asteraceae: 1) *Cirsium* spp. (thistle); 2) *Taraxacum* spp. (dandelion); and 3) unknown Asteraceae. Similarly, Fabaceae could be divided into 1) *Lotus* sp. (probably birdsfoot trefoil, *Lotus corniculatus* L.); 2) *Medicago* sp. (all which was probably alfalfa, *Medicago sativa* L.); and 3) unknown Fabaceae. Dicot pollen grains that could not be identified were grouped in an "unknown" category. We found two types of monocot pollen (based on pollen grain size), hereafter referred to as the "small" and "large" monocots. Both the small and large monocot pollen grains were of a general type (i.e., prolate and with a single sulcus), indicating that they were clearly neither grass (Poaceae) nor cattail (Typhaceae) pollen (Kapp 1969).

Pollen provisioned by *Megachile relativa*. Fifteen of 16 types of pollen distinguished occurred in *M. relativa* nests (Fig. 1). The 145 cells sampled contained a mean ($\pm$ SE) of 3.1 $\pm$ 0.1 types of pollen (range 1–7), but there was considerable variation in the number of pollen types per cell. At one extreme, there were nine cells in which we were able to find just a single pollen type among thousands of grains present in each sample. In a few cases, entire nests contained relatively few pollen types. One nest, for example, averaged just 1.3 $\pm$ 0.2 pollen types per cell (range 1–2) and two of its six cells each contained a single type (one with *Taraxacum*, the other with the large monocot). The other extreme was one particularly diverse nest with eight cells that averaged 4.8 $\pm$ 0.5 pollen types per cell (range 3–7) and contained a total of 8 different pollen types. Note that we cannot be sure that all cells in this nest were provisioned by the same female, because nest supersedure is common in trap nesters (Krombein 1967).

The *M. relativa* pollen samples came either from frass (N = 116) or uneaten provisions (N = 29). Thus, we were concerned that using different types of samples might bias results if maceration or digestion of some pollen types reduced their detection in frass. However, similarity in the prevalence of different pollen types in samples from larval frass and uneaten provisions of

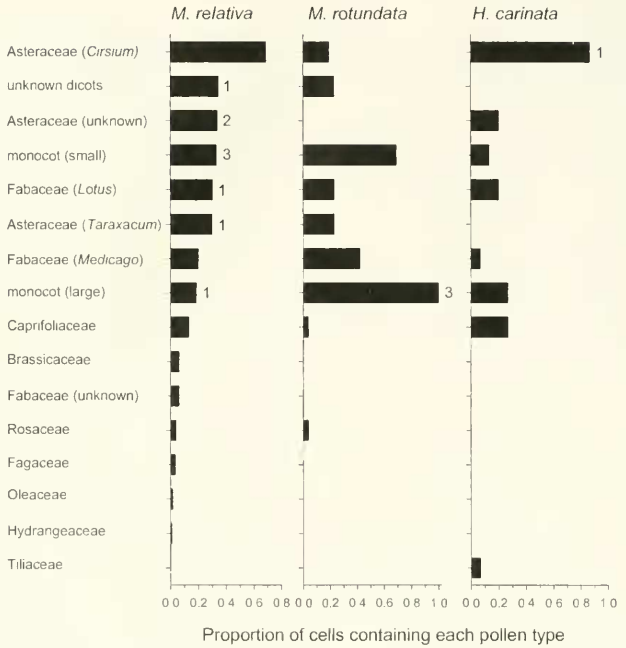

Fig. 1. Pollen records for three species of Megachilidae. Pollen types are ranked from top to bottom based on their occurrence in *Megachile relativa* records. Numbers by bars indicate the number of cells that in which the pollen type made up 100% of the provision.

M. relativa ($r = 0.73$, $N = 15$, $P = 0.002$) indicates that timing of sampling (i.e., pre- vs. post-ingestion) did not markedly affect our results. Therefore, we combined data from provision and frass samples.

The three types of Asteraceae were among the five most prevalent types in *M. relativa* cells. Strickler et al. (1996), who collected pollen samples from *M. relativa* cells in northern Michigan, found Asteraceae from a diversity of genera, including *Cirsium*, to be the most prevalent pollen types; they also found pollen of Hypericaceae, Onagraceae, and Rosaceae. Along with Asteraceae and Rosaceae, we found five additional dicot families (Caprifoliaceae, Fabaceae, Fagaceae, Hydrangeaceae, Oleaceae), as well as the two types of non-grass monocots. Along with numerous records of visitations of *M. relativa* to dicot flowers, Hurd (1979) reported *M. relativa* visits to only one monocot family (Iridaceae). We found no Iridaceae near our field sites, so the identity of the monocot pollen in our records remains a mystery. Medler and Koerber (1958) listed flowers in 23 species in 7 families visited by *M. relativa* in Wisconsin, although some of these may represent nectar-collecting rather than pollen-foraging trips.

We found considerable variation in the prevalence of different pollen types. Fagaceae and Hydrangeaceae pollen were found in small amounts in just a few cells. In addition, for some pollen types that were found in a large proportion of the cells,

there may be a few cells in which its presence in the provision was incidental (perhaps because the provisioning female used the pollen in previous cells). For example, among the 100 cells in which we identified *Cirsium* pollen (many at proportions ≥ 50%), were 7 cells in which we estimated that it made up about 5% of the pollen. Other pollen types found in only a few nests, were present in significant proportions in at least one cell. Although Oleaceae pollen was found in just two cells, it made up a minimum of 25% of the provision mass in one of these. Rosaceae pollen, though present in just six cells made up about 50% of the pollen in three cells (and approximately 90% in one of these). Both Brassicaceae and the unknown Fabaceae, present in nine cells each, were found once as approximately half of a provision mass. Caprifoliaceae pollen was found in 19 nests, but as approximately 25–50% of the provision in each. Thus, very few of the pollen types that we found can be excluded as purely incidental inclusions in *M. relativa* provisions.

The types of pollen collected in 1999 (N = 48) and 2000 (N = 97) were similar (r = 0.72, N = 15, $P = 0.002$), but when comparing WB (N = 101) and SEB (N = 44), we found no correlation between the number of cells containing particular pollen types (r = 0.20, N = 15, $P = 0.48$). This difference resulted from a higher proportion of the WB cells containing the unknown dicots (0.52 vs. 0.14; $\chi^2 = 18.3$, $P < 0.001$), the small monocot (0.45 vs. 0.2; $\chi^2 = 19.6$, $P < 0.001$), *Lotus* (0.40 vs. 0.09; $\chi^2 = 13.5$, $P < 0.001$), *Taraxacum* (0.41 vs. 0.07; $\chi^2 = 16.5$, $P < 0.001$), and the large monocot (0.25 vs. 0.05; $\chi^2 = 8.3$, $P = 0.04$). In contrast, the WB cells contained a lower proportion of the unknown Asteraceae (0.11 vs. 0.69; $\chi^2 = 78.0$, $P < 0.001$), *Medicago* (0.07 vs. 0.48; $\chi^2 = 32.7$, $P < 0.001$), and Caprifoliaceae (0.0 vs. 0.43; $\chi^2 = 50.2$, $P < 0.001$). The discrepancies in pollen prevalence between the WB and SEB samples may simply be due to differences in pollen availability between the two sites. WB is adjacent to agricultural land, whereas SEB is in a neighborhood with ornamental plants. The number of pollen types per cell at WB (mean = 3.06 ± 0.12) did not differ from the number per cell at SEB (mean = 3.02 ± 0.17; $t = 0.17$, 143 d.f.; $P = 0.87$).

Pollen provisioned by *Megachile rotundata*. In 26 *M. rotundata* cells, we identified pollen from six dicots (Asteraceae, Caprifoliaceae, Fabaceae, and Rosaceae), in addition to the two groups of monocot pollen and one unknown pollen type (Fig. 1). The *M. rotundata* nests were only several meters from two large plots of flowering alfalfa, and females were commonly seen foraging on alfalfa (Ruth P. O'Neill, personal communication). However, we found alfalfa pollen in a smaller proportion of cells (0.42) than we did the large (1.00) and small (0.69) monocot pollen; all 26 cells examined contained at least one type of monocot pollen. The cells contained a mean of 3.1 ± 0.4 types of pollen (range 1–7) and three contained only the large monocot pollen. Although Caprifoliaceae and Rosaceae pollen were each found in just one cell, the former made up 25% of the provision in the cell, whereas the latter made up approximately half of the provision. Each of the remaining pollen types made up ≥ 25% of the pollen grains in at least one cell (and often in greater proportions in numerous cells).

Although our data are based on a small sample, the results indicate a relatively wide range of pollen types provisioned by *M. rotundata*. Our records (Asteraceae, Caprifoliaceae, Fabaceae, Rosaceae, and the two monocots) partially overlap with those observed in a lowbush blueberry agroecosystem, where *M. rotundata* provisioned not only with blueberry (Ericaceae, *Vaccinium* spp.), but also Asteraceae, Rosaceae, and Salicaceae (Stubbs et al. 1994). The mix of pollen used by *M. rotundata* nesting near blueberry and alfalfa indicates that, even when presented with an overwhelming predominance of a single pollen type, this

bee includes large proportions of other pollen in its diet. Whether this mix represents a strategy of diet diversification or simply reflects some interaction between the relative availability of and preferences for different flowers remains to be determined. Although Horne (1995) demonstrated that *M. rotundata* forages for pollen on a wide variety of Fabaceae, she found that pollen preference did not correlate with success in offspring production on different pollen types. In controlled preference tests with over 200 species of 52 families, *M. rotundata* was attracted to 21 species in 7 families, with high preference for Fabaceae (including *Medicago*), Lythraceae, Crassulaceae, and Labiatae (Small et al. 1997). Several species of monocot (Liliaceae, *Allium*) were also visited, although they showed relatively low attractiveness. However, flower visitation preferences records may not necessarily coincide with pollen preferences of *M. rotundata*.

Pollen provisioned by *Heriades carinata*. *Heriades carinata* provisions included pollen from eight of the categories we distinguished, including Asteraceae, Caprifoliaceae, Fabaceae, Tiliaceae, and both types of monocot pollen (Fig. 1). Cells contained a mean of 2.1 ± 0.2 types of pollen (range 1–3). *Cirsium* was the most common pollen, occurring in 13 of 15 cells and as 100% of the pollen in one cell. Along with a larger amount of *Cirsium* pollen, one cell contained pollen of Tiliaceae (approximately 10% of the pollen) which was not found in nests of the other two bee species. Each of the other seven pollen types made up ≥ 25% of the pollen in at least one cell (and often in greater proportions in some cells). Analysis of pollen from nest cells in Michigan revealed "almost entirely" staghorn sumac (Anacardiaceae, *Rhus typhina* L.) pollen (Matthews 1965); Hurd (1979) cites 11 families of dicots visited by *H. carinata*.

Summary and Conclusions

The types of pollen used by *M. relativa*, *M. rotundata*, and *H. carinata* overlapped, which is to be expected because all three species used a variety of pollen types and had a similar local flora available. Among the 15 pollen types found in *M. relativa* nests, 9 were also found in *M. rotundata* nests and 7 in *H. carinata* nests. The larger number of pollen types found in *M. relativa* nests is likely related to the larger number of cells sampled and greater number of sites at which it was studied. For all three species, the known range of pollen in provisions is much less than the known range of flowers visited (Matthews 1965, Hurd 1979, Small et al. 1997).

It is difficult to know for particular cells whether pollen types present in low proportions represent 1) a small number of pollen-collecting trips to a particular plant species, 2) trips to flowers containing few pollen grains, or 3) incidental inclusion of a pollen type picked up during a nectar-foraging trip. Further, the proportion that represents an incidental inclusion could vary among flower types. Due to variation in flower morphology and pollen placement, some pollen types could be picked up incidentally in large quantities during nectar visits, whereas others may be transferred to the foraging bee in small numbers. Some of the pollen we identified, such as Fagaceae and Hydrangeaceae in *M. relativa* cells, may well have represented incidental inclusions of pollen picked up by nectar-foraging females. Alternatively, some of these records may represent opportunistic pollen foraging on primarily nectar-gathering trips or exploratory visits to flowers by females seeking new pollen sources. Overall, we feel that it is safe to conclude that all three species foraged for pollen on variety of plant species. A relatively wide diet breadth is especially evident for *M. relativa*, given that six of the 15 pollen types each occurred in at least one cell as pure samples, whereas two others (*Cirsium* and Rosaceae) were found as nearly pure samples in individual cells. The same can be said for the other two species where several pollen types clearly made up at least 50% of the pollen

in individual cells: 1) *Cirsium, Medicago,* the large monocot, Rosaceae, and the unknown family in *M. rotundata* cells and 2) *Cirsium, Lotus, Medicago,* the large monocot, and Caprifoliaceae in *H. carinata* cells. Nevertheless, because of uncertainties related to possible incidental inclusion of pollen, it is premature to use our records to precisely define the host ranges of these bee species, even at our sites. In addition, a complete analysis of the importance of each pollen type to the nutrition in developing bees will require estimates of individual pollen grain volume of different host species.

A potential pollinator must visit the flowers of the crop species with a degree of constancy adequate to effect high levels of pollen transfer. Even in agricultural systems, when the flowers of fruit or seed crops such as blueberry (Stubbs et al. 1994) or alfalfa predominate in close proximity to nests, *M. rotundata* may direct a high proportion of its pollen foraging trips to non-crop plant species. Horne (1995) found that *M. rotundata* exhibited only moderate preference for alfalfa relative to birdsfoot trefoil (*Lotus caniculata* L.) and crown vetch (*Coronilla varia* L.). Pollen records for *M. rotundata* suggest that control of alternative pollen sources could increase pollination efficiency in alfalfa seed crops, perhaps reducing the number of bees needed for commercial purposes. However, the types of pollen gathered by bees must be of nutritional quality adequate to sustain populations of the pollinator. Horne (1995) showed that pollen preference did not always correlate with reproductive success for *M. rotundata* given access to 11 species of plants. Thus, its moderate success on alfalfa relative to sainfoin and red clover suggests that increasing pollen source diversity could increase bee populations in agroecosystems, a goal potentially in conflict with that of increasing pollination efficiency on alfalfa. In addition, control of alternative pollen sources may negatively impact native pollinators, as well as parasitoids and predators, that depend on the flowers.

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